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Synthesis and Antimicrobial Screening of Novel 2-Amino-4,5-Diphenyl-1-(Substituted)-1*H*-Pyrrole-3-Carbonitrile

Sandip P. Dholakia*[1], Satish A. Patel[2]

1. Shankersinh Vaghela Bapu Institute of Pharmacy, Gandhinagar, Gujarat, India.

2. S. K. Patel Institute of Pharmaceutical Science & Research, Kharva, Guajarat, India.

ABSTRACT

Tuberculosis, due to its relentless nature, is now a major public health threat. The concomitant resurgence of TB with the MDR- or XDR-TB and HIV/AIDS pandemic has exposed the frailties of the current drug armatorium. Based on good structural similarity between BM-212, a novel antimycobacterial agent undergoing clinical trials, and 1,4,5 trisubstituted Pyrrole-3-Carbonitrile, we have designed novel 2-Amino-4,5-Diphenyl-1-(Substituted)-1*H*-Pyrrole-3-Carbonitrile All the compounds were screened for their antimycobacterial activity on mycobacterium tuberculosis using *H37Rv* strain by 1% proportion method. Some of the synthesized compounds exhibited potent antimycobacterial activity with MIC values in the range of 12.5-100 µg/mL.

Key words: Anti-tubercular, Pyrrole-3-Carbonitrile

*Corresponding Author Email: dholu_1984@yahoo.co.in

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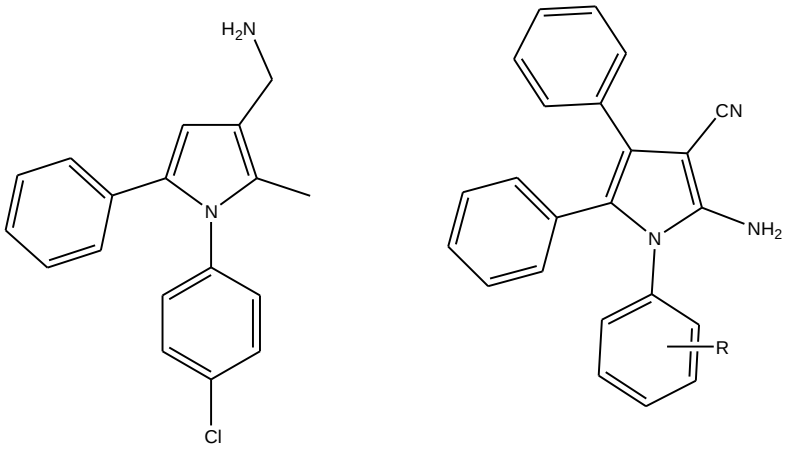
INTRODUCTION

Tuberculosis (TB), caused by Mycobacterium tuberculosis, is a disease rich in paradoxes. Currently, one third of the world population is latently infected with TB bacteria.[1] Despite the availability of the BCG vaccine and chemotherapy, TB still remains a leading infectious disease globally, especially in Third World countries. According to estimates of the World Health Organization (WHO), TB is now the leading infectious cause of death worldwide and there were an estimated 9.2 million new cases of TB every year[1] (afflicting mostly the young and productive adults). Because of relentless spread of TB throughout the world, WHO took the unprecedented step of declaring TB a global emergency in 1993 that has to be given prime importance.[2] The problem has worsened primarily due to the growing human immunodeficiency virus (HIV) epidemic and the emergence of drug resistance.[3] There were an estimated 1.5 million deaths from TB in HIV negative people and 0.2 million among people infected with HIV. The approach to chemotherapy of TB is very different from that for other bacterial infections. The organism has a long generation time and a capacity for dormancy, when its low metabolic activity makes it a difficult therapeutic target.[4-6] In addition, M. tuberculosis may be located in pulmonary cavities, empyema pus, or solid caseous material, where penetration of antibiotics is difficult or the pH is sufficiently low to inhibit the activity of most antibiotics.[7-8] Although TB can be cured by an optimized regimen comprising of various first line and second line drugs,[9-10] the emergence of MDRTB and extremely drug resistant TB (XDR-TB, first reported in November 2005[11] has created new challenges to control and defeat the disease. The concomitant resurgence of TB with the MDR-TB or XDR-TB and HIV/AIDS pandemic has exposed the frailties of the current drug armatorium.



BM-212 **Targeted compound**

Figure 1: comparison of BM-212 and targeted compound

All these suggest that there is an urgent need of new potent therapeutic agents which can be effective against resistant strains of mycobacteria. BM-212, an pyrole derivative[12] has generated considerable excitement with its antitubercular potency and presently is undergoing clinical trials. Due to structure similarity, it was thought of interest to increase the lipophilicity by incorporation of more lipophilic bulky groups on pyrrole ring of BM-212 **(Figure 1)**.This prompted us to synthesize a series of 2-Amino-4,5-Diphenyl-1-(Substituted)-1*H*-Pyrrole-3-Carbonitrile and check its antimycobacterial potential.

MATERIAL AND METHOD

Melting points of all compounds were determined in open capillaries and are uncorrected. TLC was performed on microscopic slides (2×7.5cms) coated with Silica-Gel-G and spots were visualized by exposure to iodine vapor. UV spectra were recorded in methanol double beam UV-VIS Pharmaspect 1700 Shimadzu spectrophotometer. IR spectra of all compounds were recorded in KBr (Merck) on FT-IR 8400S Shimadzu spectrophotometer. Mass spectra were recorded on SHIMADZU LCMS 2010 EV Mass Spectrometer. 1H NMR spectra were obtained on BRUKER Advance-II 400 MHz instrument in $CDCl_3$ as solvent and chemical shift were measured as parts per million downfield from tetramethylsilane (TMS) as internal standard.

PROCEDURE FOR SYNTHESIS OF 2-AMINO-4,5-DIPHENYL-1-(SUBSTITUTED)-1*H*-PYRROLE-3-CARBONITRILE

A mixture of benzoin (2 g, 0.01 mol), the appropriate amine [aniline (0.93 g, 0.01 mol), *o*-toluidine or *m*-toulidine or *p*-toulidine (1.17 g, 0.01 mol), or *o*-anisidine or *m*-anisidine or *p*-anisidine (1.23 g, 0.01mol), or *o*-chloroaniline, or *m*-chloroaniline, or *p*-chloroaniline (1.25 g, 0.01 mol)] and conc. HCl (6–8 drops) in toluene (50 mL) was heated under reflux for 6 h and cooled. Malononitrile (0.66 mg, 0.01 mol) was added, followed by a addition of sodium ethoxide (2 mL) as catalyst and left to reflux until a solid was formed. The solvent was evaporated under reduced pressure and the residue was recrystallized from methanol to give compounds **1a-j**, respectively **(Figure 2).**

ANTI-TUBERCULAR ACTIVITY

The test compounds were subjected to screening by Lowenstein Jensen Method[13] using *H37Rv* strain of *Mycobacterium tuberculosis***.**

Lowenstein Jensen media

Composition of modified L-J media

Potassium dihydrogen phosphate	1.2 g

Magnesium sulphate	0.12 g
Magnesium citrate	0.3 g
L-asparagine	1.8 g
Glycerol/Sodium pyruvate	6.0 mL/3.6 g
Distilled water	300 mL
Malachite green (2%)	16 mL
Egg homogenate	500 mL
Benzyl penicillin (1,000,000 IU/ml)	1 Ml

RESULT AND DISCUSSION

SPECTRA FOR SYNTHESIS OF 2-AMINO-4,5-DIPHENYL-1-(SUBSTITUTED)-1*H*-PYRROLE-3-CARBONITRILE

2-amino-1,4,5-triphenyl-1*H*-pyrrole-3-carbonitrile**(SD01a) :** Yellow crystalline solid ; Mass *m*/*z* (% abundance) 335 [M^+] (11.9 %) ; IR (cm^{-1}) 3566, 3641 (NH_2), 2202 (CN) ; ^{1}H NMR (ppm) 5.04 (br.s, 2H, NH_2, D_2O exchangeable), 7.04–7.67 (m, 15H, Ar-H)

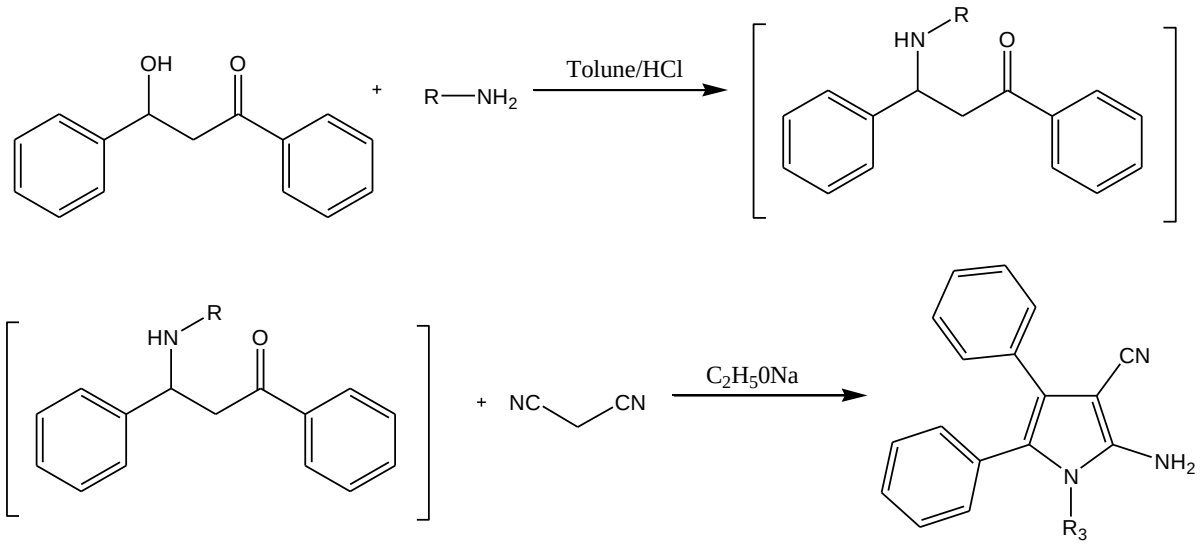
2-amino-4,5-diphenyl-1-0-tolyl-1*H*-pyrrole-3-carbonitrile**(SD01b) :** Light yellow crystalline solid ; Mass *m*/*z* (% abundance) 349 [M^+] (100 %), 350 [M^++1] (34.8 %); IR (cm^{-1}) 3438, 3315 (NH_2) 2209 (CN) ; ^{1}H NMR (ppm) 2.3 (s, 3H, CH_3), 6.07 (br.s, 2H, NH_2, D_2O exchangeable), 7.04–8.03 (m, 14H, Ar-H)

2-amino-4,5-diphenyl-1-m-tolyl-1*H*-pyrrole-3-carbonitrile**(SD01c) :** Light yellow crystalline solid ; Mass *m*/*z* (% abundance) 349 [M^+] (100 %), 350 [M^++1] (29.8 %); IR (cm^{-1}) 3441, 3315 (NH_2) 2210 (CN) ; ^{1}H NMR (ppm) 2.26 (s, 3H, CH_3), 5.09 (br.s, 2H, NH_2, D_2O exchangeable), 6.5–7.9 (m, 14H, Ar-H)

2-amino-4,5-diphenyl-1-p-tolyl-1*H*-pyrrole-3-carbonitrile**(SD01d) :** Light orange crystalline solid; Mass *m*/*z* (% abundance) 349 [M^+] (100 %), 350 [M^++1] (26.8 %); IR (cm^{-1}) 3449, 3319 (NH_2) 2215 (CN)

2-amino-1-(2-methoxyphenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile**(SD01e) :** Orange amorphous solid ; Mass *m*/*z* (% abundance) 365 [M^+] (25.3 %) ; IR (cm^{-1}) 3526, 3659 (NH_2) 2205 (CN) 1509 (C–O) ; ^{1}H NMR (ppm) 3.79 (s, 3H, OCH_3), 5.1 (br.s, 2H, NH_2, D_2O exchangeable), 6.6–7.8 (m, 14H, Ar-H)

2-amino- 1- (3- methoxyphenyl) -4,5- diphenyl- 1*H*- pyrrole-3- carbonitrile **(SD01f) :** Orange crystalline solid ; Mass *m*/*z* (% abundance) 365 [M^+] (29.6 %) ; IR (cm^{-1}) 3610 (NH_2) 2215 (CN) 1510 (C–O)



Where R= C_6H_5NH-, 2-CH_3-C_6H_5NH-, 3-CH_3-C_6H_5NH-, 4-CH_3-C_6H_5NH-, 2-OCH_3-C_6H_5NH-, 3-OCH_3-C_6H_5NH-, 4-OCH_3-C_6H_5NH-, 2-Cl-C_6H_5NH-, 3-Cl-C_6H_5NH-, 4-Cl-C_6H_5NH-

Figure 2: Reaction scheme for synthesis of designed compounds

Table 1: Physical properties of 2-Amino-4,5-Diphenyl-1-(Substituted)-1*H*-Pyrrole-3-Carbonitrile

Comp.Code	R	Molecular Formula	Molecular Weight	Melting Point (°C)	% Yield	R_f
SD01a	C_6H_5NH-	**C23H17N3**	**335.4**	170-172	80	0.14*
SD01b	2-CH_3-C_6H_5NH-	**C24H19N3**	**349.16**	228-230	70	0.32*
SD01c	3-CH_3-C_6H_5NH-	**C24H19N3**	**349.16**	136-138	90	0.35*
SD01d	4-CH_3-C_6H_5NH-	**C24H19N3**	**349.16**	170-172	60	0.27**
SD01e	2-OCH_3-C_6H_5NH-	**C24H19N3O**	**365.15**	174-177	70	0.34*
SD01f	3-OCH_3-C_6H_5NH-	**C24H19N3O**	**365.15**	164-166	85	0.37*
SD01g	4-OCH_3-C_6H_5NH-	**C24H19N3O**	**365.15**	123-125	65	0.26*
SD01h	2-Cl-C_6H_5NH-	**C23H16ClN3**	**369.85**	181-183	75	0.12*
SD01i	3-Cl-C_6H_5NH-	**C23H16ClN3**	**369.85**	160-162	70	0.44*
SD01j	4-Cl-C_6H_5NH-	**C23H16ClN3**	**369.85**	136-138	60	0.35*

Mobile Phase - *Hexane(H) : Ethyl acetate(EA) (1:1)

- **Ethyl acetate(EA) : Methanol(M) (6 : 4)

2-amino-1-(4-methoxyphenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile**(SD01g) :** Light orange amorphous solid ; Mass *m/z* (% abundance) 365 [M^+] (5.6 %) ; IR (cm^{-1}) 3660 (NH_2) 2225 (CN) 1509 (C–O)

2-amino-1-(2-chlorophenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile**(SD01h) :** Orange amorphous solid ; Mass *m/z* (% abundance) 370 [M^+] (96.3 %), 372 [$M^+ + 2$] (5.3 %) ; IR (cm^{-1})

3626 (NH_2) 2190 (CN) ; ^{1}H NMR (ppm) 5.5 (br.s, 2H, NH_2, D_2O exchangeable), 6.6–7.8 (m, 14H, Ar-H)

2-amino-1-(3-chlorophenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile**(SD01i) :** Light Orange amorphous solid ; Mass *m*/*z* (% abundance) 370 [M^+] (95.3 %), 372 [$M^+ + 2$] (4.3 %) ; IR (cm^{-1}) 3635 (NH_2) 2195 (CN)

2-amino-1-(4-chlorophenyl)-4,5-diphenyl-1*H*-pyrrole-3-carbonitrile**(SD01j) :** White amorphous solid; Mass *m*/*z* (% abundance) 370 [M^+] (93.3 %), 372 [$M^+ + 2$] (6.3 %) ; IR (cm^{-1}) 3610 (NH_2) 2190 (CN).

Table 2: Antitubercular activity of the targeted compounds

Sr. No.	Compound No.	Concentration of compound		
		12.5 µg/mL	50 µg/mL	100 µg/mL
1	**SD01a**	No growth	No growth	No growth
2	**SD01b**	Growth detected	Growth detected	No growth
3	**SD01c**	Growth detected	Growth detected	No growth
4	**SD01d**	Growth detected	Growth detected	No growth
5	**SD01e**	Growth detected	Growth detected	Growth detected
6	**SD01f**	Growth detected	Growth detected	No growth
7	**SD01g**	Growth detected	Growth detected	No growth
8	**SD01h**	Growth detected	Growth detected	No growth
9	**SD01i**	Growth detected	Growth detected	No growth
10	**SD01j**	Growth detected	No growth	No growth

CONCLUSION

All the test compounds were screened for their antimycobacterial screening by L. J. method using $H_{37}R_V$ strain. All compounds were screened at 12.5, 50, and 100 µg/mL concentration. All compounds were found to be active at 100 µg/mL except compound – **SD1e** of all compounds screened. Compound – **SD01a** was found to be active at all concentration so its MIC will be <12.5 µg/mL. These results reveal that unsubstituted aryl has more potent than substituted aryl at N_1-(Substituted)-1*H*-Pyrrole-3-Carbonitrile, so their MIC may be in between 50-100 µg/mL.

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